LWT - Food Science and Technology 82 (2017) 342–353

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Gluten reduction in beer by hydrodynamic cavitation assisted brewing of barley malts

Lorenzo Albanese [a], Rosaria Ciriminna [b], Francesco Meneguzzo [a, *], Mario Pagliaro [b, **]

[a] Istituto di Biometeorologia, CNR, via G. Caproni 8, 50145 Firenze, Italy
[b] Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy

ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form
26 March 2017
Accepted 19 April 2017
Available online 22 April 2017

Keywords:
Beer
Brewing
Gluten
Hydrodynamic cavitation
proline

ABSTRACT

While gluten content in beers can be quite toxic to coeliac patients as well as to the broader group of gluten-intolerant people, using gluten-free raw ingredients leads to severe deprivation of flavor and taste, as well as other existing methods to lower the gluten concentration are still generally not firmly established as well as quite costly. During the development and test of a novel brewing technology based on controlled hydrodynamic cavitation, early evidence arose of gluten reduction in wort and finished beer from 100% barley malt, in correspondence with suitable cavitation regimes during both mashing and fermentation. Experimental tests are reviewed and discussed, while few hypotheses are advanced, pointing to the degradation of proline residues, the most recalcitrant among gluten constituents, leading to gluten concentration reduction in the unfermented wort and/or during fermentation and maturation, the latter due to the enhanced proline assimilation by yeasts. Direction for further research includes at least repetition of experiments and design of new ones, extension of the range of cavitation regimes, and identification of strict operational parameters as functions of brewing recipes.

1. Introduction

With nearly 200 billion liters per year, beer is the alcoholic beverage most widely consumed around the world (Amienyo & Azapagic, 2016). Its basic ingredients, *i.e.* water, malt or grains, hops and yeasts, and production methods have barely changed over centuries beyond obvious technological improvements and ingredients diversification (Ambrosi, Cardozo, & Tessaro, 2014; Pires & Brányik, 2015), while knowledge of the respective microbiological processes is well established (Bokulich & Bamforth, 2013).

Despite a moderate dietary beer consumption is considered a healthy attitude under certain conditions (de Gaetano et al., 2016), the gluten content, arising from barley and wheat malts and grains from which most beers are produced, make that beverage unsuitable for consumption by coeliac disease patients (Hager, Taylor, Waters, & Arendt, 2014).

Contrary to most other inflammatory disorders, both genetic precursors and exogenous environmental factors triggering the coeliac disease are known since long enough, along with its basic mechanisms (Sollid, 2002). It develops in susceptible patients because of their intolerance to ingested fractions of cereal proteins referred collectively as gluten, including proteins of barley (hordein), wheat (gliadin) and rye (secalin). In particular, the gluten epitopes recognized by the immune system in the human intestine are generally very rich in proline and glutamine residues, which are amino acids and gluten components. Proline residues, showing high levels in barley (Deželak, Zarnkow, Becker, & Košir, 2014; Malalgoda & Simsek, 2016), were observed to play a key role by means of their multiple ways of influencing the immunogenicity of gluten peptides (Balakireva & Zamyatnin, 2016).

Production and marketing of *very low gluten content* (<100 mg/L) or *gluten-free* (<20 mg/L) beers is still in its starting phase and the projected market value in Europe is estimated on the order of several billion Euros per year (Harasym & Podeszwa, 2015).

Most gluten-free beers foresees the use of at least a fraction of malts derived from cereals and pseudo-cereals not containing gluten or its precursors, such as sorghum, buckwheat, quinoa, amaranth (Wijngaard & Arendt, 2006; de Meo et al., 2011), maize and oat (Yeo & Liu, 2014). Nevertheless, the respective brewing techniques for cereals different from barley have not yet been well

Abbreviations: BIAB, Brew In A Bag; CN, Cavitation Number; FAN, Free Amino-Nitrogen; GRAS, Generally Recognized As Safe; HC, Hydrodynamic Cavitation; SG, Silica Gel.

* Corresponding author.
** Corresponding author.
E-mail addresses: f.meneguzzo@ibimet.cnr.it (F. Meneguzzo), mario.pagliaro@cnr.it (M. Pagliaro).

http://dx.doi.org/10.1016/j.lwt.2017.04.060
0023-6438/

established, despite some recent encouraging results (Mayer et al., 2016).

Alternatively, generally complex and costly techniques are sometimes used, such as filtration and enzymatic ones, aimed at conditioning the malts in order to boost the processes leading to the precipitation of proteins, in particular polypeptides, during mashing, fermentation and possibly stabilization (Dostálek, Hochel, Méndez, Hernando, & Gabrovská, 2006; Hager et al., 2014). Beyond uncertainties, complexity and costs, finished beers most often fall far away from traditional aroma and flavor customers are used to. An alternative technique consists in the use of silica gel (SG) at some stage of the brewing process, mainly fermentation, in order to selectively remove proteins, without practically affecting both valuable yeast nutrients such as free amino-nitrogen (FAN) and foam-causing proteins (Benítez, Acquisgrana, Peruchena, Sosa, & Lozano, 2016). Although SG is recognized as a safe food additive both in US and Europe, its use adds to cost and process complexity.

Fermentation, usually lasting several days since the pitching of yeast strains in the cooled and aerated wort, is the most important brewing step for the gluten reduction in traditional beers. During fermentation, assimilation of fermentable sugars, amino acids, minerals and other nutrients occurs along with metabolic production of ethanol, CO_2, higher alcohols, esters and other substances (Bokulich & Bamforth, 2013; Landaud, Latrille, & Corrieu, 2001; Pires & Bányik, 2015). In particular, amino acids accumulated in the fermenting wort supply nearly all the nitrogen needed by the yeasts' cellular biosynthesis in the form of FAN, as well as affect bitterness, flavor and foam stability (Choi, Ahn, Kim, Han, & Kim, 2015). Among amino acids, most important is glutamine, a gluten component, along with other ones belonging to the so called "Group A" which undergo the fastest assimilation by yeasts' cells at a rate depending on the specific yeast strain (Pires & Bányik, 2015): glutamine assimilation and transformation explains the fall of gluten concentration during fermentation. Once Group A amino acids are assimilated, other ones belonging to Groups B and C are more gradually and slowly assimilated until nitrogen-deprived residuals from original amino acids are turned into higher (fusel) alcohols and esters, strongly impacting beers' flavor. An only amino acid belongs to Group D, namely proline, whose assimilation by yeast cells was deemed negligible until few years ago (Lekkas, Stewart, Hill, Taidi, & Hodgson, 2005). However, more recently the proline itself, whose concentration in the fermenting wort can be quite high, was found to lead to the formation of fusel alcohols, therefore impacting beer's aroma, flavor and overall alcohol content (Procopio, Krause, Hofmann, & Becker, 2013). The proline assimilation rate revealed a high sensitivity to the yeast strain, increasing in high stress conditions due to the shortage of more easily assimilated amino acids, as well as a positive dependence on the availability of molecular oxygen, which is a scarce resource during anaerobic fermentation.

Given the fast assimilation of glutamine, practically the gluten concentration in the wort as well as in the finished beer will depend on the proline assimilation rate, which, along with its role about gluten toxicity, makes its assimilation, degradation and further reduction during fermentation and maturation—the latter lasting several weeks either in dedicated vessels or in bottles —very beneficial to the food safety of the finished beers.

This study shows early evidence of the potential for brewing of conventional barley malts assisted by controlled hydrodynamic cavitation (HC) to reduce the gluten concentration in the respective beers by means of suitable cavitation regimes and operational parameters, *i.e.* by purely electro-mechanical means, without either changing ingredients or using additives as well as any other technological pathway.

2. Materials and methods

2.1. Brewing unit

A dedicated equipment was built from known or commonly available commercial components, in order to investigate the effects of hydrodynamic cavitation processes upon gluten concentration.

Fig. 1 shows the experimental installation, including a closed hydraulic loop with total volume capacity around 230 L, powered by a centrifugal pump (Lowara, Vicenza, Italy, model ESHE 50–160/75 with 7.5 kW nominal mechanical power) with open impeller 0.174 m in diameter. Rotation speed was set around 2900 rpm. As shown in the manufacturer's technical documentation at page 48 ("Serie e-SH (in Italian), "2016), the maximum pressure and volumetric flow rate were around 4 atm and 1500 L min^{-1}, respectively.

A Venturi tube, with the same geometry described in detail in Fig. 2(B) of a previous study by the authors (Albanese, Ciriminna, Meneguzzo, & Pagliaro, 2015), is used as the cavitation reactor and preferred over an orifice plate since it was observed that orifices are quickly obstructed by the circulating solid particles.

The design allows for upscaling of a single installation unit up to the order of 10,000 L, for housing further pumps and cavitation reactors, and for straightforward integration of isolated components, such as pumps and HC reactors, into existing brewing and fermentation plants of virtually any size.

All but one of the tests designed to study the HC effects upon the gluten concentration ran in brew-in-the-bag (BIAB) mode, where the malts are not allowed to circulate, being caged in the cylindrical vessel shown in Fig. 1, in turn made up of a stainless steel fine grid with a perforated pipe arranged along the vessel axis, connected to the same external pump used for thermal stabilization. In BIAB tests, malt milling before mashing was required and performed by means of a small semiautomatic stainless steel roller mill. On the contrary, hops—being pitched after the removal of the cylindrical

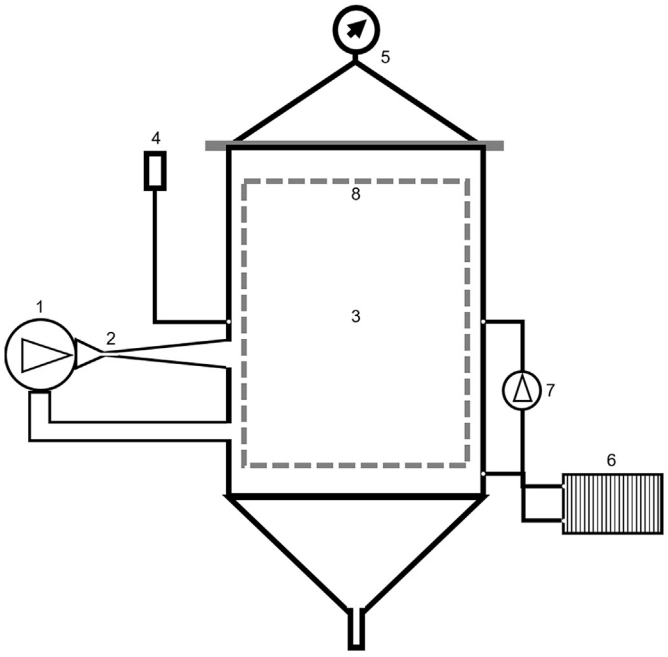

Fig. 1. Simplified scheme of the experimental HC-based installation. 1—centrifugal pump, 2—HC reactor, 3—main vessel, 4—pressure release valve, 5—cover and pressure gauge, 6—heat exchanger, 7—circulation pump, 8—malts caging vessel. Other components are commonly used in state-of-the-art hydraulic constructions.